Global Change Biology

Global Change Biology (2010) **16**, 1046–1056, doi: 10.1111/j.1365-2486.2009.02007.x

Greater seed production in elevated CO_2 is not accompanied by reduced seed quality in *Pinus taeda* L.

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Abstract

For herbaceous species, elevated CO_2 often increases seed production but usually leads to decreased seed quality. However, the effects of increased atmospheric CO_2 on tree fecundity remain uncertain, despite the importance of reproduction to the composition of future forests. We determined how seed quantity and quality differed for pine trees grown for 12 years in ambient and elevated (ambient + $200\,\mu L\,L^{-1}$) CO_2, at the Duke Forest free-air CO_2 enrichment (FACE) site. We also compared annual reproductive effort with yearly measurements of aboveground net primary productivity (ANPP), precipitation (P), potential evapotranspiration (PET) and water availability [precipitation minus potential evapotranspiration (P−PET)] to investigate factors that may drive interannual variation in seed production. The number of mature, viable seeds doubled per unit basal area in high-CO_2 plots from 1997 to 2008 ($P < 0.001$), but there was no CO_2 effect on mean seed mass, viability, or nutrient content. Interannual variation in seed production was positively related to ANPP, with a similar percentage of ANPP diverted to reproduction across years. Seed production was negatively related to PET ($P < 0.005$) and positively correlated with water availability ($P < 0.05$), but showed no relationship with precipitation ($P = 0.88$). This study adds to the few findings that, unlike herbaceous crops, woody plants may benefit from future atmospheric CO_2 by producing larger numbers of seeds without suffering degraded seed quality. Differential reproductive responses between functional groups and species could facilitate woody invasions or lead to changes in forest community composition as CO_2 rises.

Keywords: fecundity, germination, *Pinus taeda*, reproduction, reproductive allocation

Received 17 March 2009 and accepted 18 May 2009

Introduction

The effect of rising atmospheric CO_2 on seed production has important consequences for future forest composition (Garbutt & Bazzaz, 1984; LaDeau & Clark, 2001, 2006a; Ibáñez *et al*., 2006). Insufficient seed supply can limit seedling recruitment in forests, particularly given the low probability of a seed successfully developing into a seedling (Clark *et al*., 1998; Caspersen & Saprunoff, 2005). Because many species produce more seeds at high CO_2 (Jackson *et al*., 1994; Jablonski *et al*., 2002), forests may be less limited by seed supply in the future.

However, increased seed numbers produced at high CO_2 are often correlated with reduced seed quality, as measured by seed nitrogen concentration, viability and/or weight (Andalo *et al*., 1996; Ward & Strain, 1997; Huxman *et al*., 1999; Jablonski *et al*., 2002; Thürig *et al*., 2003; He *et al*., 2005). Because developing seeds can represent a large carbon sink for the plant (Fenner, 1986; Wardlaw, 1990; Zamski, 1995; Bazzaz, 1997; Huxman *et al*., 1999), elevated CO_2 can also lead to heavier seeds (Hussain *et al*., 2001; Thürig *et al*., 2003; Darbah *et al*., 2008). Increased carbon allocation to seeds can be matched by complementary nutrient allocation, resulting in no change in the seed nutrient content, as is often the case in legumes (Miyagi *et al*., 2007). Alternatively, seed quality will decline if nutrient allocation cannot keep pace with the greater carbon allocation

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(i.e., Huxman *et al*., 1999; Jablonski *et al*., 2002; Thürig *et al*., 2003; He *et al*., 2005). While reduced seed quality with increased seed production at high CO_2 is common in herbaceous species, such a response may be less common in woody species. For example, Stiling *et al*. (2004) found increased acorn production, but no change in acorn mass or viability, for *Quercus* species grown at $704\,\mu L\,L^{-1}$ CO_2.

Here, we investigate the long-term effect of elevated CO_2 on the fecundity of loblolly pine grown at the Duke Forest free-air CO_2 enrichment (FACE) site. Previous studies at this site found that young *Pinus taeda* L. trees grown in high CO_2 (ambient + $200\,\mu L\,L^{-1}$) produced more seeds per unit basal area than ambient-grown trees (LaDeau & Clark, 2001, 2006a); these same trees had 91% heavier seeds with three times greater germination success than seeds from ambient-grown trees (Hussain *et al*., 2001). However, reproductive trees were scarce in this young stand in 1998 when seed quality was initially assessed (LaDeau & Clark, 2001). It is therefore still unclear whether CO_2 enrichment can enhance both seed number and quality as trees age and produce larger numbers of seed cones, or if the effects seen in earlier studies are transient and associated with young stands. If long-term increases in *P. taeda* fecundity are associated with greater seed quality, the overall CO_2 effect on recruitment success could be substantial. We therefore tested the hypothesis that both long-term seed quantity and quality (including mass, viability and nutrient content) are increased by elevated CO_2 in this woody species.

Although seed production is greater at high CO_2, there is considerable interannual variation in fecundity at the Duke Forest FACE site (LaDeau & Clark, 2006a, b). In years when regional seed production by loblolly pines was reduced, individual fecundity was low in trees from both ambient and elevated CO_2 (LaDeau & Clark, 2006a). However, it is unclear how interannual variability in reproduction is related to overall biomass production or environmental conditions. Interannual variation in fecundity may be caused by variation in stand productivity with no change in the relative investment of resources allocated to reproduction between years. Alternatively, in years where environmental stress limits productivity, trees may reduce their proportional allocation to reproduction (e.g., by aborting seeds). To evaluate these ideas, we used aboveground net primary productivity (ANPP) data to examine the relationship between stand productivity and reproductive effort (as indicated by seed production) and test the hypotheses that (1) interannual variation in seed production is correlated with ANPP and (2) the percent of ANPP invested in reproduction is constant across years of varying ANPP. Additionally, because much of the interannual variation in ANPP at this site is driven by water availability (H. R. McCarthy *et al*., unpublished data), we investigated the relationship between seed production and precipitation (P), potential evapotranspiration (PET) and water availability [precipitation minus potential evapotranspiration (P−PET)] at this site.

Materials and methods

Study site

The Duke Forest FACE experiment is located in the Piedmont region of North Carolina (Orange County, 35.97°N, 79.09°W). In August 1996, FACE technology was installed in a 13-year-old unmanaged loblolly pine (*P. taeda* L.) plantation (Hendrey *et al*., 1999). *P. taeda* at this site are even-aged, maternal half-siblings and accounted for 98% of the basal area in 1996. The replicated experiment consists of six 30 m diameter circular plots of forest surrounded by 32 vertical pipes that deliver an ambient or elevated CO_2 (ambient + $200\,\mu L\,L^{-1}$ CO_2) atmosphere. Three plots receive elevated CO_2.

P. taeda is a monoecious species. Mature trees produce both female and male strobili, with the production of female strobili generally beginning several years earlier. Seed cones develop slowly and seeds are not dispersed until 2 years after reproductive buds are initiated; seed filling occurs in the final year. Few *P. taeda* trees were reproductively mature at the initiation of the FACE experiment in 1996. By 1998, seed cones were only observed on 2% of ambient and 4% of CO_2 fumigated trees (LaDeau & Clark, 2001); this increased to 26% and 43%, respectively, in 2000. Additionally, the number of mature seeds collected from ground traps has increased from 24 seeds in all six plots in 1997 (12 seeds from high-CO_2 plots) to a maximum of 1339 seeds in all six plots in 2007 (855 from high-CO_2 plots). Our analysis therefore focuses on seeds collected after 1999, when most of the trees became reproductively active.

Seed trap collections

Seeds were collected monthly in ground traps from 1997 to 2008. Beginning in May 1997, twelve $0.18\,m^2$ traps, located randomly within each of the six plots, were sampled monthly; in 2004, these were replaced with twelve $0.218\,m^2$ traps per plot. Traps were constructed from baskets lined with screen and covered with chicken wire to deter squirrel predation. Beginning in 1999, seed mass was recorded for each *P. taeda* seed collected. Between 1997 and 2002, data collection focused only on mature pine seeds (filled seeds >10 mg fresh weight) with no visible predation damage. Since 2002, seeds with evidence of predation (predated seeds) have been tallied as a separate category. As of 2003, counts were recorded for immature seeds; these were usually flat in appearance and empty when dissected. Starting in 2007, the data collection was further expanded to include the weight of male catkins from seed traps. Lastly, between 1997 and 2003, the weights of all reproductive parts of loblolly pine (cones, pieces of cones, seeds, and catkins) that fell into ground traps were collected.

The mean annual number of seeds collected from ground traps within each plot was divided by the mean annual basal area for each plot (see ''Methods'') to account for treatment-induced differences in relative stand density. For germination determinations, a random sample of mature seeds collected from seed traps in 2000 were soaked in water, stratified, and then germinated as described below.

Cone samples

Cone samples were taken in 2000 and 2003. In September 2000, seed cones were sampled from trees before ripening as per Barnett (1976) and Sirius (2000): cones were collected before scale opening when cone moisture content was $<70\%$ (specific gravity $= 0.88$), as determined from a sample of cones from outside the plots. In the elevated CO_2 plots, 42 cones were collected from 13 trees that were chosen for cone presence and accessibility. Cones were less accessible in ambient CO_2 plots, so six cones collected from the ambient plots were supplemented with 30 additional cones collected from 16 trees located just outside of the ambient plots. Ambient trees produced only half the total cones that elevated CO_2 trees produced in 2000 (505 vs. 1034 cones observed), and the majority of these were in the upper portion of the canopy. All cones were collected over a period of 2 days and stored in paper bags for several weeks to ripen. When scales opened, cones were shaken to remove seeds and the few cones with unopened scales were dried at 38 °C for 9 h to induce opening. A total of 5551 seeds were collected. We recorded cone weight, total seed weight per cone and mean seed weight per cone.

All seeds were de-winged and submerged in water for 48 h. Seeds that remained floating after 2 days were assumed to be empty and were removed (i.e., Barnett, 1976); 64% and 44% of seeds sampled from ambient and elevated plots, respectively, were unfilled. Remaining seeds were stratified for 120 days at 10 °C (Hussain *et al.*, 2001). Following stratification, 300 random seeds from both ambient and elevated CO_2 trees (100 seeds per plot) were transferred to Petri dishes (25 seeds $dish^{-1}$) lined with damp filter paper, placed in the dark at room temperature and checked daily for germination over 28 days. Upon radicle emergence, individual seeds were placed in 7 cm plastic tubes (1 cm radius) filled with damp, sterile cotton, placed in a glasshouse and watered daily. No fertilizers were added, hence seedling growth was a function of light availability (constant across treatments) and stored seed reserves. Survival and total seedling growth (root + shoot length) were evaluated 80 days after seeds were removed from stratification.

Additional cone sampling directly from trees was constrained by limitations on destructive sampling in the plots. Following a windstorm in fall 2003, cones were collected from the ground in the six plots; 18 and 22 cones were collected from ambient and elevated CO_2 plots, respectively. The above procedures were followed for seed extraction, germination and seedling growth. Only filled seeds with no obvious predation were counted (71 and 114 seeds from ambient and elevated CO_2 plots). Unlike in 2000, seeds that remained floating after 48 h were not removed from the experiment. A random sample of seeds that failed to germinate after 28 days was cut open to evaluate gametophytic integrity. Survival and seedling growth were quantified 30 days after seeds were removed from stratification. Cone weight and the number of seeds per cone were not analyzed in 2003 due to signs of squirrel predation on some cones.

Nutrient analyses

Carbon and nitrogen content were assessed on seeds from cones harvested in 2000 and seeds collected in ground traps in 2003. Seed tissue (megagametophyte and embryo) was separated from the seed coat and frozen for one week at −80 °C. In 2000, tissue from 10 random seeds per plot was pooled for analysis. In 2003, seeds were weighed and sorted into two samples and analyzed independently (1) five seeds per plot weighing 10.0–20.0 mg, and (2) five seeds per plot weighing >20.0 mg. Two aliquots of homogenized tissue from each sample (5–10 mg each) were analyzed for percent dry mass elemental carbon and nitrogen (CE Instruments NC 2100 elemental analyzer, Thermoquest Italia, Milan, Italy). To compare seed traits from the FACE stand with seeds from more mature trees, we collected pine seeds from 30 ground traps in an 80-year-old mixed pine-hardwood stand (HW) located 50 m from the FACE plots in both years. Seed weight and nutrient content were quantified for the HW sample as above.

Basal area and ANPP

To help account for differences in seed production caused by differences in tree size, basal area was calculated from annual diameter measurements, determined with dendrometer bands. From 1996 to 2003, dendrometer bands were present on 31–34 pines per plot (Moore *et al.*, 2006); in spring 2004, bands were added to all pines. During the period in which only a subset of trees had dendrometer bands, the diameters of unbanded trees were calculated using the relationship between individual tree basal area and basal area increment derived from banded trees within each plot and each year (H. R. McCarthy *et al.*, unpublished data). Basal area in elevated CO_2 plots was 5% greater than ambient plots in 1996 before CO_2 fumigation, but 36% greater by 2004.

Heights of trees bearing dendrometer bands were measured three times during the experiment (end of growing season for 1996, 2001 and 2006) with either a height pole or a survey laser (Criterion 400, Laser Technology Inc., Englewood, CO, USA). Together with diameter measurements, these height measurements were used to estimate aboveground biomass based on scaling equations of stems and branches (Naidu *et al.*, 1998; Fang *et al.*, 2000). The difference in stem and branch biomass between consecutive years was added to foliage production (McCarthy *et al.*, 2007) to obtain estimates of ANPP (H. R. McCarthy *et al.*, unpublished data). Conversions of ANPP from biomass to carbon were made using 0.48 for stems, branches and foliage (Oren *et al.*, 2001; Schäfer *et al.*, 2003). To compare seed number data with these ANPP values, the number of mature seeds collected from litter traps between 1997 and 2008 was converted to a $\mathrm{g\,C\,m^{-2}\,yr^{-1}}$ basis for each plot by multiplying the annual seed number on a ground area basis by the mean seed mass and %C data for the appropriate CO_2 treatment. These values for annual mature seed production from each plot (in $\mathrm{g\,C\,m^{-2}}$ ground area $\mathrm{yr^{-1}}$) were then divided by the ANPP (in $\mathrm{g\,C\,m^{-2}\,yr^{-1}}$) for each plot from the same year and multiplied by 100% to estimate the percentage of ANPP invested in mature seed.

Atmospheric variables

Precipitation (P) measurements at the site were made above the canopy in one ring (plot 4) with a tipping bucket (model TI, Texas Instruments, Austin, TX, USA). Potential evapotranspiration values were taken from adjusted pan evaporation measurements made at a weather station about 7 km from the experiment. Atmospheric data are from 1999 to 2005 for the growing season only; this is normally from April to September, but data collection varies among years depending on actual growing season length.

Statistical analyses

We used generalized linear mixed models with normal (i.e., seed mass) and Poisson (i.e., seeds in seed traps) error distribution to estimate the CO_2 effect on repeated measures data. Plot, trap and year were treated as random effects, while CO_2 treatment and pine basal area were estimated as fixed effects. Results are reported as means ± SE. Statistical analyses were implemented in *R* version 2.7.2 (R Foundation, from http://www.r-project.org) and JMP 7.0.1 (SAS Institute, Cary, NC, USA).

Results

Seed production and seed mass

Mean mature seed production per unit pine basal area was on average two times greater in elevated than in ambient CO_2 between 1999, when trees became reproductive, and 2008 (generalized linear model, $Z = 6.42$, $P < 0.001$; Fig. 1a and Table 1). The number of mature seeds collected in traps was significantly and positively related to pine basal area (coefficient = 0.03, $P = 0.002$) and the interaction term for CO_2 treatment × basal area was significantly negative (coefficient = −0.02, $P = 0.001$), indicating that each incremental increase in basal area had a greater impact on the fecundity of ambient than high-CO_2 grown trees. The magnitude of the CO_2 effect also varied significantly among years (Fig. 1); high CO_2 increased mean mature seed production by as little as 18% in 2002, but as much as 146% in both 1999 and 2000.

When we examined data from 2003 to 2008, when immature and predated seeds were also recorded, we found no CO_2 effect on the number of predated seeds (Table 1), though the number of predated seeds was higher in years and plots where more mature seeds were also collected. Predated seeds accounted for between 5.6% (in 2003) and 48.6% (in 2002) of the combined number of mature and predated seeds in ambient plots; these values were 7% (in 2006) and 37.7% (in 2002) in elevated CO_2 plots. This same pattern holds for immature seeds: more immature seeds were collected from sites with more mature seed production (repeated measures analysis, coefficient = 0.001, $P < 0.001$), but high CO_2 did not change the proportion of total seeds that were immature. The ratio of total (mature + predated + immature) pine seeds to mature pine seeds within each plot was similar between CO_2 treatments and years (Fig. 2; analysis of variance, CO_2 treatment effect $P = 0.21$).

Unlike the increase in mature seed production, mature seed mass did not differ significantly between ambient and elevated CO_2 treatments (Fig. 3, Table 1; repeated measures analysis, CO_2 treatment effect $P = 0.13$). Seed mass did vary by year (repeated measures analysis, among-year variance = 2.84); mean annual seed weight ranged from 15.7 ± 1.8 mg in 2004 to 23.7 ± 1.8 mg in 1999.

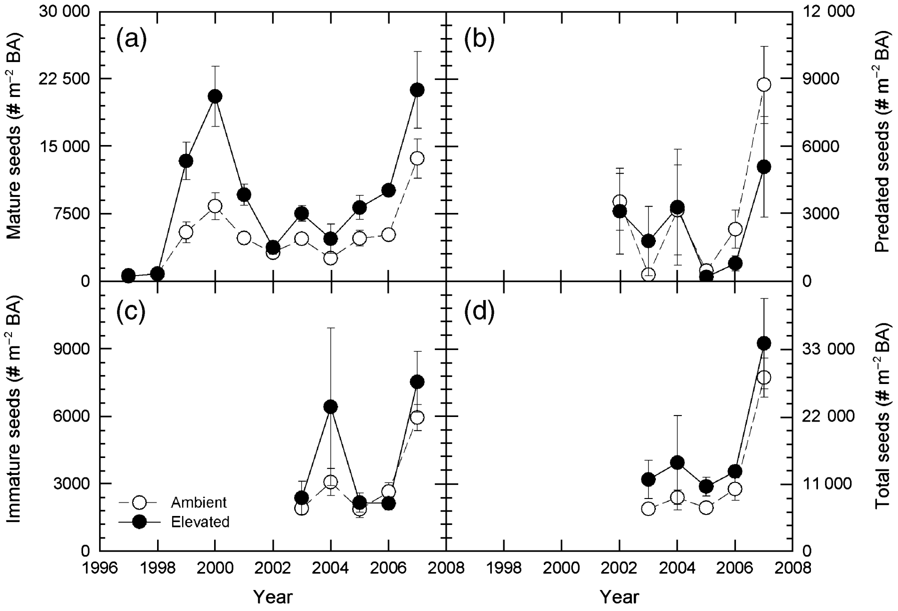

Fig. 1 Annual number of pine seeds per m^2 pine basal area in ambient (open symbols, dashed line) and elevated (filled symbols, solid line) CO_2 plots. (a) Mature seeds; (b) predated seeds; (c) immature seeds; (d) total seeds (mature + predated + immature). Means ± SE, $n = 3$ plots.

Table 1 Reproductive responses to elevated CO_2 in loblolly pine

	Ambient	Elevated	E/A	*P*-value
Seed quantity				
Mature seeds (# m^{-2} BA yr^{-1}) (1999–2008)	4918 ± 1097	9145 ± 2113	1.86	<0.001
Predated seeds (# m^{-2} BA yr^{-1}) (2002–2008)	3089 ± 1257	2379 ± 739	0.77	ns
Immature seeds (# m^{-2} BA yr^{-1}) (2003–2008)	3086 ± 750	4125 ± 1179	1.34	<0.1
Total seeds (# m^{-2} BA yr^{-1}) (2003–2008)	12 257 ± 4062	16 726 ± 4341	1.36	ns
Seed quality				
Mass (mg) – Seed traps (1999–2008)	21.7 ± 0.9	21.9 ± 1.0	1.00	ns
Mass (mg) – Cones – 2000	22.3 ± 1.8	25.1 ± 2.1	1.16	ns
Mass (mg) – Cones – 2003	21.0 ± 0.58	21.0 ± 1.2	1.00	ns
% Carbon – 2000	55.6 ± 0.6%	55.6 ± 0.3%	1.00	ns
% Carbon – 2003	51.8 ± 0.7%	50.6 ± 0.4%	0.98	ns
% Nitrogen – 2000	7.6 ± 0.4%	7.7 ± 0.1%	1.01	ns
% Nitrogen – 2003	7.6 ± 0.1%	7.4 ± 0.1%	0.97	ns
C : N – 2000	7.40 ± 0.35	7.24 ± 0.13	0.98	ns
C : N – 2003	6.84 ± 0.15	6.92 ± 0.20	1.01	ns
Germination – 2000	21%	31%	1.48	ns
Germination – 2003	24%	25%	1.04	ns
Seedling growth (mm) – 2000	66.3 ± 3.9	88.2 ± 1.2	1.33	ns
Seedling growth (mm) – 2003	14.2 ± 2.2	17.6 ± 2.3	1.24	ns
Seedling survival – 2000	55%	69%	1.25	ns
Seedling survival – 2003	100%	100%	1.00	ns
Estimated reproductive effort				
Mature pine seeds (# m^{-2} yr^{-1})	23.3 ± 4.6	47.5 ± 9.2	2.04	<0.001
Total pine seeds (# m^{-2} yr^{-1})	44.4 ± 7.1	81.5 ± 14.0	1.84	n/a
Pine cones (# m^{-2} yr^{-1})	0.75 ± 0.12	1.20 ± 0.21	1.60	n/a
Pine cones (g m^{-2} yr^{-1})	15.8 ± 2.5	25.2 ± 4.4	1.60	n/a
Pine catkins (g m^{-2} yr^{-1})	189.2 ± 54.0	427.1 ± 170.4	2.26	ns

Means ± SE, except for germination and survival.

E/A, ratio of the elevatedtoambient CO_2 response; ns, nonsignificant result ($P > 0.05$); n/a, nonapplicable where results were scaled to estimate reproductive effort.

Measurements on a pine basal area basis are indicated as m^{-2}BA; values on a ground area basis are indicated as m^{-2}.

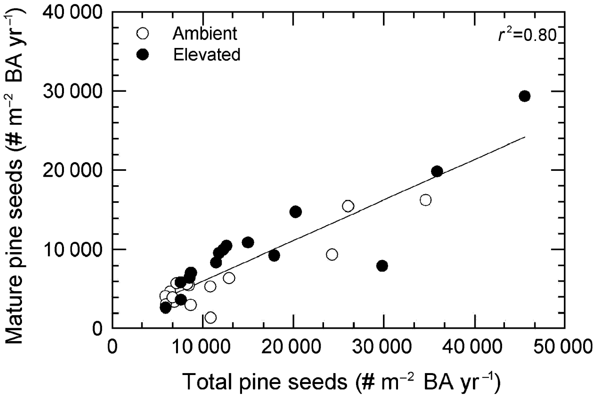

Fig. 2 Relationship between the annual number of total (mature + predated + immature) and mature pine seeds per m^2 pine basal area in ambient (open) and elevated (filled) CO_2 plots. Regression: $y = 858.8 + 0.51x$ ($P < 0.01$).

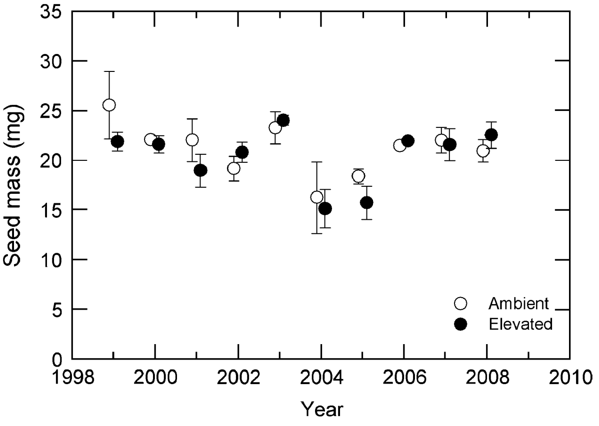

Fig. 3 Annual mean mass of mature pine seeds from ambient (open) and elevated (filled) CO_2 plots. Means ± SE, $n = 3$ plots.

Analyses of seeds and cones from cone collections in 2000 and 2003 do not generally indicate a large CO_2 effect on seed quality. Cone mass ranged from 12.7 to 39.8 g in CO_2-fumigated plots and 12.5 to 33.4 g in ambient plots in 2003 (Table 1). The mean number of seeds per cone in 2000 was 59 ± 5 for ambient samples and 68 ± 5 for fumigated samples (two-sided *t*-test, $P > 0.1$). There was no CO_2 effect on mean seed mass from cones in either 2000 or 2003 (two-sided *t*-tests, $P > 0.1$; Table 1).

We estimated the investment in female reproductive output using mature seed production, the ratio of total seeds to mature seeds, the mean number of seeds per cone, and the mean cone mass for each CO_2 treatment (Table 1). Cone production varied from 6.4 to 45.5 $g\,m^{-2}\,yr^{-1}$ in ambient plots and 9.4 to 63.8 $g\,m^{-2}\,yr^{-1}$ in elevated CO_2 plots, an increase of >40% in both cases. Mean male reproductive effort in 2007 and 2008, as measured by catkin mass in ground traps, varied from 135.2 to 243.3 $g\,m^{-2}\,yr^{-1}$ in ambient plots and 256.7 to 597.5 $g\,m^{-2}\,yr^{-1}$ in elevated CO_2 plots (Table 1). There was no significant difference in the weight of catkins collected in litter traps between CO_2 treatments over the 2 years (analysis of variance, $P = 0.15$).

Seed germination

Seeds collected in ground traps in 2000 from ambient plots had slightly, but nonsignificantly, lower germination success than high-CO_2 seeds (Table 1; two-sided *t*-test, $P > 0.1$). Of the 300 ambient CO_2 seeds stratified from the cone collections in 2000, only 20 germinated (6.6%) vs. 83 of 300 seeds from cones grown in high CO_2 (27%). Variability among plots was considerable, ranging from 0% to 69% germination success. For unknown reasons, complete germination failure occurred in samples from one elevated CO_2 plot. The CO_2 treatment was not a significant factor in the seed germination (analysis of variance, CO_2 treatment: $F_{1,4} = 0.035$, $P > 0.05$).

Seeds harvested from cone collections in 2003 had similar germination success in both CO_2 treatments (Table 1; analysis of variance, CO_2 treatment: $F_{1,4} = 0.066$, $P > 0.05$). Among-plot variability in germination ranged from 13% to 46%. Seeds that failed to germinate in 2003 were all either empty or contained shriveled gametophytic tissue. After seeds were stratified, they germinated in an average of 12.3 days, though germination times ranged from 1 to 38 days. There was no CO_2 effect on the rate of germination in either 2000 or 2003 (χ^2 from Kaplan–Meier survivorship analysis, $P > 0.05$).

Seedling growth and survival

Of the seeds that germinated in 2000, 50% from ambient plots and 48% from elevated plots developed into seedlings (18 and 80 of total seedlings, respectively). A smaller proportion of seedlings from seeds collected in ambient plots survived the 60-day experiment relative to elevated CO_2 seedlings (Table 1). The final mean length of surviving seedlings after 60 days was similar for both CO_2 treatments (Table 1; two-sided *t*-test, $P > 0.1$).

In 2003, 50 and 75 of the germinated seeds from ambient and high-CO_2 plots, respectively, were grown for 30 days. All seedlings survived the 30-day experiment. By comparison, 15% of total seedling mortality in 2000 occurred in the first 30 days (9% of ambient and 27% of high-CO_2 seedling mortality). Mean seedling length after 30 days was slightly, but not significantly, smaller in the parental ambient CO_2 samples (Table 1; two-sided *t*-test, $P > 0.1$).

Nutrient analyses

We found no CO_2 effect on the carbon-to-nitrogen (C : N) ratio of seed tissue in either 2000 or 2003 (Table 1; analysis of variance, CO_2-by-year: $F_{1,13} = 1.16$, $P > 0.05$). Seed weight in 2003 (10–20 mg vs. >20 mg) did not affect the C : N ratios, so data were pooled across weights. Samples collected from FACE rings did not differ from C : N ratios of *P. taeda* seeds collected in a nearby mature forest in either year (means 7.37 and 6.83 in 2000 and 2003, respectively). There was no difference in either year in the percent nitrogen or carbon by weight in seed tissue from ambient and fumigated plots (Table 1).

ANPP and interannual variation in reproduction

Interannual variation in mature seed production varied synchronously with annual ANPP (Fig. 4). There was no difference in the relationship between ANPP and mature seed production between CO_2 treatments (analysis of variance, CO_2 by ANPP, $P = 0.57$). Production of mature pine seeds increased with increasing ANPP (Fig. 5a; analysis of variance, $P = 0.0001$). The investment of ANPP in mature pine seed varied from only 0.009% to 0.11%; there was no significant relationship between ANPP and the percent of ANPP allocated to seed production (Fig. 5b; analysis of variance, $P = 0.59$). Unlike seed production, the mass of all reproductive structures collected in ground traps (including seeds, catkins, cones and cone fragments) was not positively correlated with ANPP (Fig. 5c; analysis of variance, $P = 0.31$), although there was significantly more reproductive mass in elevated CO_2 plots ($P = 0.032$).

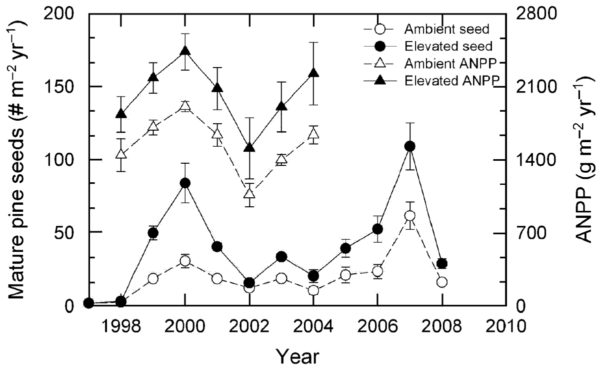

Fig. 4 Interannual variation in mature pine seed production (per m^2 ground area) and aboveground net primary productivity (ANPP) in ambient (open symbols) and elevated CO_2 (filled symbols) plots. Means ± SE, $n = 3$ plots.

While interannual variation in ANPP is correlated with water availability (P−PET) at this site (McCarthy *et al.*, 2006a; H. R. McCarthy *et al.*, unpublished data), annual seed production across both CO_2 treatments was more strongly correlated with PET (analysis of variance, $r^2 = 0.18$, $P = 0.0012$) than with either P−PET ($r^2 = 0.09$, $P = 0.028$) or P ($r^2 < 0.001$, $P = 0.88$). The number of mature seeds was negatively correlated with PET (Fig. 6a). While the PET × CO_2 interaction for seed production was not quite statistically significant (analysis of variance, $P = 0.052$), there was a trend for elevated CO_2 plots to respond more strongly to changes in PET than ambient CO_2 plots (Fig. 6a; ambient CO_2: slope $= -10.3$,

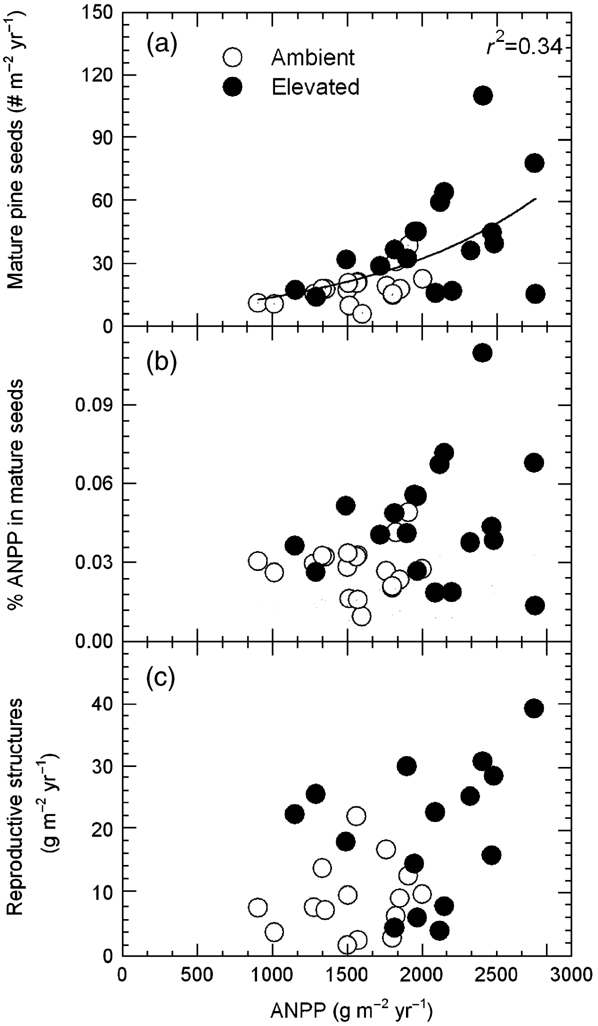

Fig. 5 Relationship between aboveground net primary productivity (ANPP) and: (a) mature pine seed production per m^2 ground area, (b) the percentage of ANPP invested in mature pine seed and (c) total reproductive structures (pine cones, catkins, seeds) per m^2 ground area in ambient (open symbols) and elevated (filled symbols) CO_2 plots from 1999 to 2004. Regression: (a) $y = 6.15e^{0.000834x}$.

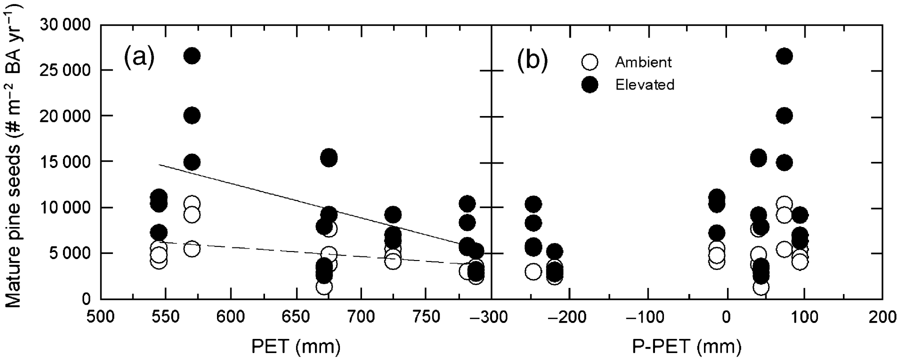

Fig. 6 Relationship between annual mature pine seed production per m^2 pine basal area and: (a) annual growing season potential evapotranspiration (PET), (b) annual growing season water availability (P−PET) in ambient (open symbols, dashed line) and elevated CO_2 (filled symbols, solid line) plots from 1999 to 2004. Regressions: (a) ambient CO_2: $y = -10.3x + 11\,812$, $r^2 = 0.18$; elevated CO_2: $y = -37.6x + 35\,253$, $r^2 = 0.32$.

$r^2 = 0.18$, $P = 0.052$; elevated CO_2: slope $= -37.6$, $r^2 = 0.32$, $P = 0.008$). The number of mature seeds produced was positively correlated with P−PET (Fig. 6b); there was no P−PET $\times$ CO_2 interaction (analysis of variance, $P = 0.18$) and regressions of P−PET on seed production for each CO_2 treatment were not significant (analysis of variance, ambient CO_2: $P = 0.21$, elevated CO_2: $P = 0.067$).

Discussion

Seed quantity and quality will be important determinants of population growth and sustainability in future forest communities. Results from numerous herbaceous studies suggest that increased seed numbers at elevated CO_2 may be met with decreased seed size and/or seed quality (Jackson *et al.*, 1994; Huxman *et al.*, 1999; Jablonski *et al.*, 2002; Thürig *et al.*, 2003). Here we present evidence that, unlike many herbaceous species, *P. taeda* can increase carbon allocation to seed numbers with no effect on seed mass, nutrient content, or viability. This is consistent with observations from the few other studies examining reproductive responses to elevated CO_2 in trees. Young *P. taeda* trees increased seed production per unit basal area under high atmospheric CO_2 (LaDeau & Clark, 2001, 2006a); consistent with this earlier finding, we found that each incremental increase in plot-level basal area had a greater impact on fecundity in ambient trees than high-CO_2 grown trees.

Although CO_2-induced increases in seed production have often been matched with reduced seed quality in herbaceous plants (e.g., Jablonski *et al.*, 2002), this does not seem to hold true for woody species. Acorn production in scrub-oak was between four to seven times greater at elevated than ambient CO_2, but there was no change in the individual acorn mass (Stiling *et al.*, 2004). Similarly, growth at elevated CO_2 led to an 85% greater number of fruits in sour orange trees over 17 years without affecting individual fruit mass (Kimball *et al.*, 2007). The ability to increase seed quantity without reducing seed quality may therefore be a key difference between the response of herbaceous and woody plants to elevated atmospheric CO_2. If this is true, greater propagule pressure from woody species could contribute to increases in woody encroachment in herbaceous and grassland communities as atmospheric CO_2 rises.

While we have concentrated on seed production and quality, we also investigated male reproductive output. The mass of male catkins collected in ground traps was more than doubled over 2 years in elevated CO_2 plots compared with ambient plots, although the result was not statistically significant. An increase in male reproductive effort would agree with previous research that showed more pollen cones were produced per unit basal area by *P. taeda* in elevated than ambient CO_2 plots from 2001 to 2005 (LaDeau & Clark, 2006b). Because neither pollen cone length (and presumably therefore cone mass) nor the number of pollen grains per cone was affected by CO_2 (LaDeau & Clark, 2006b), the doubling in total mass of male reproductive structures presented here likely represents a doubling of pollen production in elevated CO_2 plots.

Seed quality

Our analysis of ground trap collections demonstrates no effect of CO_2 treatment on seed mass. The decline in mean seed weight in 2004 and 2005 corresponded to low seed production in those years and may be related to weather conditions at any point during the 2 years between cone initiation and seed dispersal (e.g., Schultz, 1997; Cain & Shelton, 2000), including drought and an ice storm in 2002 (McCarthy *et al.*, 2006b). While ground traps might capture seeds from trees growing outside the experimental plots, we believe this to be of minimal importance due to the short, dense canopies at these sites; the low seed numbers in 1997 and 1998, before most experimental trees reached reproductive maturation, supports this view. Additionally, our cone harvest experiments support the ground trap analyses. Mean seed mass, germination rates and seedling characteristics in 2000 and 2003 were all greater for seeds from cones grown in high CO_2, though never statistically different from ambient samples. Variation among seeds within plots (or among individual cones) among years was consistently greater than any CO_2 enrichment effect. Similar responses have been observed in seed mass of paper birch, which was significantly increased in only 1 out of 3 years in elevated CO_2 trees, while germination rates of seed produced at high CO_2 were significantly higher in 2 of 3 years (Darbah *et al.*, 2008).

Our results differ from the increased seed mass and quality found for a small number of seeds early in the experiment in 1998 (Hussain *et al.*, 2001). By 1999, the mean mass of seeds was unaffected by CO_2 treatment, and germination, seed nutrient content and seedling survivorship were also similar between CO_2 treatments. While the apparent disappearance of a CO_2 enrichment response in seed quality could indicate photosynthetic acclimation to high CO_2, we think this is unlikely. If photosynthetic acclimation had reduced carbon availability during our study period, we might expect to see concurrent down-regulation of a CO_2 effect on fecundity. Although interannual variation was large, we found no evidence for a decrease in seed production or seed mass over time. As well, there is no evidence of photosynthetic down-regulation in current year needles at this site; while elevated CO_2 does induce some acclimation in 1-year-old needles in these trees, the stimulation of photosynthesis by the high-CO_2 environment still results in a 40% increase in net photosynthesis (Crous *et al.*, 2008; Maier *et al.*, 2008). The absence of CO_2 enrichment effects in our 2000 and 2003 seed germination and nutrient results may therefore not be due to a reduced CO_2 response (vs. Hussain *et al.*, 2001), but instead likely reflect increased fecundity and changes in maturation status between these studies. The increase in the total number of trees contributing seed in the plots between 1998 and 1999 was considerable (LaDeau & Clark, 2001). By 2003, seed production was relatively high in the majority of trees growing at high CO_2, while a few ambient trees had emerged as exceptional seed producers (LaDeau & Clark, 2006a).

The absence of a reduction in nitrogen content in high-CO_2 seeds suggests that these trees are either not nitrogen-limited (e.g., Springer *et al.*, 2005) or that they have shifted allocation of nitrogen preferentially to seed production. Previous work at this site has shown that elevated CO_2 trees have increased nitrogen uptake rates (Finzi *et al.*, 2007), which could account for their ability to sustain seed nitrogen concentrations at levels equivalent to ambient trees, despite doubling seed production. However, there may also be a degree of increased nitrogen allocation to reproduction, given that growth at the site is nitrogen-limited (McCarthy *et al.*, 2006a; Palmroth *et al.*, 2006).

Interannual variation in reproduction

This is the first paper, to our knowledge, to link long-term interannual variation in seed production with yearly changes in stand net primary productivity. The correlation between seed number and current year ANPP could be driven by resource limitations that concurrently induce failure at the seed filling stage and limit primary productivity. This possibility is supported by our finding that the mass of the total reproductive structures (which mainly consists of cones and cone pieces) is not related to ANPP. Because cones are initiated 2 years before seed filling is completed, seed number will more strongly reflect environmental limitations in the year the seeds are filled than will total reproductive mass. Although NPP at the Duke FACE site is primarily driven by differences in nitrogen availability between plots, soil water availability and storm damage are more important drivers of interannual variation in stand productivity (McCarthy *et al.*, 2006a, b; H. R. McCarthy *et al.*, unpublished data). Similarly, we found that seed production was lower in years with greater PET and years with lower water availability. This agrees with work showing fewer loblolly pine cones produced in years with early summer drought (Langdon, 1979).

Many species reduce reproductive investment when environmental conditions are poor, although few studies have been reported in woody species. For two species of Mediterranean oak (*Quercus ilex* and *Q. humilis*), the ability to ripen existing acorns was linked to water stress: the percent of initiated acorns that matured decreased as the strength of summer drought increased (Espelta *et al.*, 2008). Thus, lower seed production in plots and years where PET was high (or P−PET was low) likely result from seed abortion during years with poor climate conditions, as opposed to greater investment in seeds in favorable years. The inability to increase reproductive allocation when productivity is high agrees with conclusions from a calcareous grassland, where the increase in reproduction at elevated CO_2 was similar to the increase in biomass, implying a constant allocation to seed production (Thürig *et al.*, 2003). A meta-analysis of elevated CO_2 studies also found no significant change in reproductive allocation in crops and wild plants (Jablonski *et al.*, 2002). Unlike differences in the response of seed quality to CO_2, the invariance of reproductive allocation with changing CO_2 may therefore be similar between herbaceous and woody life-forms, allowing us to effectively link growth responses to reproduction. For example, invasive species show much greater increases in biomass with rising CO_2 than crops or trees (Ziska, 2003); if reproductive allocation remains a constant fraction of biomass, invasive species will have not only a competitive growth advantage over native species, but also a competitive advantage in seed production.

Differences in the magnitude of CO_2 response between species will have important consequences for future community composition. Increased production of high-quality seeds by woody species in response to rising CO_2 would give them a reproductive advantage over herbaceous species that produce more seeds but cannot maintain seed quality. This may facilitate woody encroachment into herbaceous communities, a widespread phenomenon that has already been linked to rising CO_2 (Bond & Midgley, 2000; Davis *et al.*, 2007). Within functional groups, species that exhibit pronounced growth responses to high CO_2 should further enhance their competitive ability by also producing more seeds due to constant reproductive allocation. Forest community composition may therefore be expected to shift towards those species that most effectively take advantage of rising CO_2 to enhance their reproductive output.

Acknowledgements

The authors thank Ines Ibáñez, Jeff Pippen, Will Cook and Melissa Chernick for assistance with data collection and nutrient analysis, and FACE site managers (including W. H. Schlesinger, G. R. Hendrey, K. F. Lewin and J. Nagy) for operating the FACTS-1 experiment. This research was made possible by a Sigma Xi Grant-In-Aid, Duke University Biology Department Grant-In-Aid, NSF grants DEB 9453498 and 0235425, and US Department of Energy, Office of Energy Research, Grant DE-FG0595ER62083. D. A. W. was funded by the US Department of Energy, Program in Ecosystem Research and a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship. S. L. L. was supported in part by a NSF Predoctoral Research Fellowship and by an American Fellowship from the American Association of University Women.

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